

5    zearalenone, cytochalasin, griseofulvin, ergochrome,  
      cercosporin, marticin, xanthocillin, coumarins,  
      tricothecenes, fusidanes, sesterpenes, amatoxins,  
      malformin A, phallotoxins, pentoxin, HC toxin, psilocybin,  
10    bufotenine, lysergic acid, sporodesmin, pulcheriminic  
      acid, sordarins, fumonisins, ochratoxin A, and fusaric  
      acid.

      With some certain embodiments of aspects of the  
      invention, the secondary metabolite is a modulator of cell  
      surface receptor signaling or a biosynthetic intermediate  
15    thereof. The term "cell surface receptor" is as used  
      before. Modulators of cell surface receptor signaling  
      might function by one of several mechanisms including,  
      without limitation, acting as agonists or antagonists,  
      sequestering a molecule that interacts with a receptor  
20    such as a ligand, or stabilizing the interaction of a  
      receptor and molecule with which it interacts. Preferred  
      modulators of cell surface signaling include, without  
      limitation, the insulin receptor agonist L-783,281 and the  
      cholecystokinin receptor antagonist asperlicin.

25       In certain embodiments of aspects of the invention,  
      the secondary metabolite is a plant growth regulator or a  
      biosynthetic intermediate thereof. A "plant growth  
      regulator" is a molecule that controls growth and  
      development of a plant by affecting processes that  
30    include, without limitation, division, elongation, and  
      differentiation of cells. Preferred plant growth  
      regulators include, without limitation, cytokinin, auxin,  
      gibberellin, abscisic acid, and ethylene.

      In certain embodiments of aspects of the invention,  
35    the secondary metabolite is a pigment or a biosynthetic  
      intermediate thereof. A "pigment" is a substance that  
      imparts a characteristic color. Preferred pigments  
      include, without limitation, melanins and carotenoids.

      In certain embodiments of aspects of the invention,  
40    the secondary metabolite is an insecticide or a  
      biosynthetic intermediate thereof. An "insecticide" is a  
      molecule that is toxic to insects. Preferred insecticides  
      include, without limitation, nodulisporic acid.

5 In certain embodiments of aspects of the invention,  
the secondary metabolite is an anti-neoplastic compound or  
a biosynthetic intermediate thereof. An "anti-neoplastic"  
compound is a molecule that prevents or reduces tumor  
formation. Preferred anti-neoplastic compounds include,  
10 without limitation, taxol (paclitaxel) and related  
taxoids.

The phrase "increased activity" is used herein to  
refer to a characteristic that results in an augmentation  
of the inherent negative or positive function of the  
15 regulatory protein.

The invention provides variant regulator proteins of  
secondary metabolite production with increased activity  
and methods of producing the same. The invention further  
provides for the identification of specific amino acid  
20 residues that are important to the functioning of  
secondary metabolite regulator proteins. By way of non-  
limiting example, variant regulator proteins of the  
secondary metabolite regulator lovE are presented herein.

As known to those skilled in the art, certain  
25 substitutions of one amino acid for another may be  
tolerated at one or more amino acid residues of a wild-  
type regulator protein absent a change in the structure,  
activity and/or function of the wild-type protein. Such  
substitutions are referred to in the art as "conservative"  
30 substitutions, and amino acids may be categorized into  
groups that identify which amino acids may be substituted  
for another without altering the structure and/or function  
of the protein.

As used herein, the term "conservative substitution"  
35 refers to the exchange of one amino acid for another in  
the same conservative substitution grouping in a protein  
sequence. Conservative amino acid substitutions are known  
in the art and are generally based on the relative  
similarity of the amino acid side-chain substituents, for  
40 example, their hydrophobicity, hydrophilicity, charge,  
size, and the like. In a preferred embodiment,  
conservative substitutions typically include substitutions  
within the following groups: Group 1: glycine, alanine,

5 and proline; Group 2: valine, isoleucine, leucine, and  
methionine; Group 3: aspartic acid, glutamic acid,  
asparagine, glutamine; Group 4: serine, threonine, and  
cysteine; Group 5: lysine, arginine, and histidine; Group  
6: phenylalanine, tyrosine, and tryptophan. Each group  
10 provides a listing of amino acids that may be substituted  
in a protein sequence for any one of the other amino acids  
in that particular group.

As stated *supra*, there are several criteria used to  
establish groupings of amino acids for conservative  
15 substitution. For example, the importance of the  
hydropathic amino acid index in conferring interactive  
biological function on a protein is generally understood  
in the art (Kyte and Doolittle, *Mol. Biol.* **157**:105-132  
(1982)). It is known that certain amino acids may be  
20 substituted for other amino acids having a similar  
hydropathic index or score and still retain a similar  
biological activity. Amino acid hydrophilicity is also  
used as a criteria for the establishment of conservative  
amino acid groupings (see, e.g., U.S. Patent No.  
25 4,554,101).

Information relating to the substitution of one amino  
acid for another is generally known in the art (see, e.g.,  
Introduction to Protein Architecture : The Structural  
Biology of Proteins, Lesk, A.M., Oxford University Press;  
30 ISBN: 0198504748; Introduction to Protein Structure,  
Branden, C.-I., Tooze, J., Karolinska Institute,  
Stockholm, Sweden (January 15, 1999); and Protein  
Structure Prediction: Methods and Protocols (Methods in  
Molecular Biology), Webster, D.M. (Editor), August 2000,  
35 Humana Press, ISBN: 0896036375).

In one embodiment of the first aspect, the invention  
provides an improved regulator protein comprising an amino  
acid sequence coding for a variant of the lovE protein  
having at least one specific mutation that gives rise to  
40 greater transcription-activating properties of the  
regulator protein and/or increased lovastatin synthesis.

By way of non-limiting example, certain amino acid  
residues and mutations thereof in the lovE regulatory